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A SYSTEM FOR EFFICIENT GENETIC ANALYSES USING PCR FOLLOWED BY %LIGASE% -MEDIATED GENE DETECTION AND VISUALIZATION BY TIME-RESOLVED FLUORESCENCE
SAMIOTAKIS M; PARIK J; KWIATKOWSKI M; LAGERQUIST A; PETTERSSON U; %LANDEGREN U%
DEP. MED. GENET., UPPSALA UNIV. SWED., UPPSALA, SWED.
PROCEEDINGS OF THE 8TH INTERNATIONAL CONGRESS OF HUMAN GENETICS, WASHINGTON, D.C., USA, OCTOBER 6-11, 1991. AM J HUM GENET 49 (4 SUPPL.). 1991. 194. CODEN: AJHGA
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Clinical Genetics: Molecular Diagnosis *(continued)*

1036 Poster Symposium 57

DNA screening of South African hyperlipidemics for familial hypercholesterolemia and familial defective apolipoprotein B-100. M. J. Kotze*, E. Langenhoven and A. E. Retief. Dept. of Human Genetics, Faculty of Medicine, University of Stellenbosch, Tygerberg, South Africa.

Familial hypercholesterolemia (FH) and familial defective apolipoprotein B-100 (FDB) are genetic disorders associated with elevated low density lipoprotein (LDL) levels. Hyperlipidemic South African individuals were screened for specific mutations in the LDL receptor and apo B genes to determine the distribution in individuals with different lipid profiles.

Analysis of the point mutations in PCR-amplified genomic DNA has shown that the apo B mutation was absent in the hyperlipidemics studied. A large proportion of individuals, who were diagnosed as non-FH hypercholesterolemics on clinical data, carry a LDL receptor gene defect. This indicates that conventional methods for the diagnosis of FH, based mainly on lipid determinations and a family history of CHD, do not always allow an accurate diagnosis of the disease. Screening of hyperlipidemic individuals for specific LDL receptor gene mutations can provide a definite diagnosis of FH, which may lead to better counselling and optimal treatment.

1037

Genetic analysis of the atrial natriuretic factor gene in familial orthostatic intolerance. M.R.S. Krishnamani*, John A. Phillips III, M.L. Summar, I. Biaggioni, V.F. Haile and R.M. Robertson, Vanderbilt University School of Medicine, Nashville, TN.

Atrial natriuretic factor (ANF) is a small peptide hormone synthesized by atrial cardiomyocytes. Infusion of ANF in man causes decreased arterial pressure due to decreased cardiac output and vascular resistance and decreased intravascular volume. Individuals with familial orthostatic intolerance (FOI) develop hypotension and dizziness on standing and may have mitral valve prolapse. Interestingly, individuals in certain FOI kindreds have paradoxical increases in ANF levels on standing suggesting possible abnormalities in ANF or in its clearance receptor. We identified an intragenic ANF PCRFLP to map ANF and study its linkage relationship to FOI. HhaI digestion of a 2.552 kb PCR fragment containing the entire ANF gene detected a RFLP whose major allele was 0.81. Using the CEPH panel of DNAs we found ANF to be linked to CRI-C52 (θ=.18, LOD = 3.77), CRI-L336 (θ=.20, LOD = 3.4) and tissue fucosidase α-L-1 (θ=.13, LOD = 4.2). In a multiplex FOI kindred with apparant autosomal recessive inheritance the 2 affected sibs and 1 non-affected sib were concordant and discordant, respectively, for maternal ANF alleles. Our data 1) confirm the assigment of the ANF locus to 1p36 and identify multiple linked markers, and 2) raise the possibility that derangements of ANF may contribute to FOI.

1038

Use of CA repeat marker DXS548 for prenatal diagnosis of Fragile X syndrome and the identification of new possibly polymorphic sites in the Fragile X region. D.P.A. Kuhl*(1), S.T. Warren (3), G.J. Riggins (3), D.L. Nelson (1) and C.T. Caskey (1,2). (1) Baylor College of Medicine and (2) Howard Hughes Medical Institute, Houston, TX, and (3) Emory University, Atlanta, GA.

Currently, diagnostic studies of Fragile X families is often difficult to the low heterozygosity of linked markers and the high rate of recombination between useful markers and the Fragile X locus. In addition, cytogenetic diagnosis of Fragile X is often complicated by the subjective interpretation required to make the diagnosis. We report here the use of a new highly polymorphic CA repeat marker for diagnostic use in several families. DXS548 was derived from a Fragile X YAC clone, RS 46, which has been shown by pulse field gel analysis to the less than 175 kb from the breakpoints of a number of somatic cell hybrids which were produced by fragmentation at the Fragile site. Recent physical mapping data indicate that the breakpoints of these cell lines are clustered extremely close to the Fragile X locus. Linkage analysis also supports the very close localization of this probe to the Fragile X locus. No recombinations have been observed between the Fragile X locus and DXS548 resulting in an inferred LOD score of greater than 40 with a θ of 0.00. This marker is a CA repeat polymorphism which is highly polymorphic and can easily and rapidly be assayed. This marker has been successfully used in several families with the probe being informative in every family tested. While this probe is quite useful, it suffers from shadow banding which is characteristic of dinucleotide repeats. We have adopted an end labeling protocol to reduce the complexity of the bands observed. To develop a marker which is easier to score, the Fragile X region, which is totally contained in YAC clone 209G4, was scanned for trinucleotide and tetranucleotide repeats. Previously reported experience indicates that these longer repeat elements are free of shadow banding. We have identified regions that contain such repeats and are in the process of characterizing them.

1039

Strand separating gel electrophoresis (SSGE): detection of polymorphisms useful in family studies. D.Labuda, E. Zietkiewicz, D. Sinnett, Ch. Richer, J. Michaud, G. Mitchell, and M. Vanasse. Génétique Médicale, Département de Pediatrie, Université de Montréal, Centre de Recherche, Hôpital Ste-Justine, Montréal, Québec, Canada H3T 1C5.

Many point mutations can be detected due to electrophoretic mobility difference in native gels between allelic DNA fragments that were rendered single stranded. Although this approach has been mainly exploited to look for mutations causing genetic disorders, it can simultaneously be applied to detect neutral polymorphisms which are tightly linked to the mutated locus and can serve as genetic markers for segregation analysis in families at risk. For example, in many cases of Duchenne muscular dystrophy (DMD), it is difficult to distinguish between deletion carriers and normal female family members because routine Southern hybridizations and PCR-amplifications are not quantitative. However, if a female at risk is shown heterozygous for a neutral polymorphism in the DNA segment known to be deleted in the proband, this eliminates her risk of being a carrier of this deletion. We have shown that at least 3 out of the seven multiplex-PCR primer-pairs (Chamberlain et al. 1989), used to detect the majority of DMD causing deletions, lead to PCR-amplified DNA segments with allelic variation observed by SSGE. Thus, a direct assay of the deletion mutation and a family linkage study are integrated in a single experimental test. We applied this approach to different regions of the dystrophin gene and also to autosomal genes. We are currently sequencing these fragments of dystrophin gene which were shown to by polymorphic by SSGE. Supported by the Fonds de Recherche en Santé du Québec.

1040

Detection of deletions and intragenic RFLPs by PCR amplification in DMD/BMD patients in Singapore. PS Lai*, JSH Tay, PS Low, WL Lee, GAS Koh and GC Gan. Division of Human Genetics, Dept of Paediatrics, National University of Singapore.

The polymerase chain reaction (PCR) technique is widely used today for the diagnosis of genetic diseases. We report a study of 24 DMD/BMD children (15 Chinese, 7 Malays and 2 Indians) in Singapore using PCR amplification. Using the multiplex PCR to simultaneously coamplify 9 deletion-prone exons within the DMD gene, deletions were detected in 14 out of 24 patients (58.3%). Three exons were found to be frequently deleted. One rare case of total deletion of all 9 exons was observed. The results were confirmed by Southern hybridisation. The 10 non-deletion cases were screened for 3 intragenic RFLP markers, namely pERT 87-15/XmnI, pERT 87-15/BamHI and pERT 87-8/TaqI. Three patients were informative for two of these markers. Two of the mothers were found to be heterozygous for the BamHI and TaqI RFLP alleles. This technique of detecting partial deletions and intragenic RFLPs was successful in the molecular diagnosis of DMD/BMD in 17 out of 24 (70.8%) cases in Singapore. It will be useful as a rapid tool for prenatal diagnosis and carrier testing.

1041

A system for efficient genetic analyses using PCR followed by ligase-mediated gene detection and visualization by time-resolved fluorescence. M. Samiotakis (1), J. Parik (1), M. Kwiatkowski (2), A. Lagerquist (1), U. Pettersson (1), and U. Landegren* (1). (1) Dept. of Med. Gen., Uppsala Univ. Sweden and (2) Pharmacia Diagnostica, Uppsala, Sweden.

Routine genetic analyses in e.g. clinical settings require the detection and quantitation of specific DNA or RNA sequences and the distinction among sequence variants. We have developed a strategy to perform these analyses in a rapid and efficient standardized format. Isolated or reverse transcribed DNA molecules are amplified by PCR. In a subsequent detection step a third oligonucleotide is added to the amplification reactions and ligated to one of the PCRprimers provided there is a PCR product strand to serve as template in an oligonucleotide ligation assay (OLA). A succesful ligation will join a biotinylated oligonucleotide to one labeled with a rare earth metal chelate. The metal ions may be sensitively detected by time-resolved fluorescence. The use of different metal ions permit the simultaneous analysis of allelic sequence variants in one reaction well or the quantitative comparison with an internal control sequence. Reaction components may be stored in microtiter wells, ready for the addition of nucleic acid samples. Individual reactions are handled using a disposable manifold with an array of 96 solid supports, projecting into the microtiter wells. These steps serve to greatly reduce the potential for mix-ups and contamination and permit the rapid processing of large numbers of reactions.The assay specifically identifies 96 PCR products in about an hour with a signal to noise ratio of up to 500. We have applied the technique to the screening for infectious genomes, detection of tumor-specific RNAs and the identification of genetic variants.